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EXPERIMENTAL STUDY

Mechanism and effect of Shijueming (*Concha Haliotidis*) on serum calcium in spontaneously hypertensive rats

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Abstract

OBJECTIVE: To observe the impact of Shijueming (*Concha Haliotidis*) on spontaneously hypertensive rats via blood pressure, serum calcium, vascular smooth muscle membrane L-type calcium channel α1 C subunit (CaL-α1C), plasma membrane calcium-ATPase (PMCA) mRNA expression, and the L-type calcium channel in vascular smooth muscle cells.

METHODS: Twelve-week-old male rats with spontaneous hypertension were divided into three groups: a Shijueming (*Concha Haliotidis*) group (group 1), a nifedipine group (group 2), and a distilled water group (group 3). All were given a four-week treatment. Blood pressure and dissociative serum calcium were examined before treatment. Blood pressure was taken every week during treatment. Atomic absorption spectrometry was used to examine dissociative serum calcium. Reverse transcription-polymerase chain reaction was used to examine the expression of CaL-α1C and PMCA1 mRNA. The patch clamp technique was used to examine the electrophysiological characteristics of the vascular smooth muscle cell calcium channels.

RESULTS: After treatment, blood pressure of the Shijueming (*Concha Haliotidis*) group lowered but not significantly ($P>0.05$). Blood pressure of the nifedipine group lowered significantly ($P<0.05$). Blood pressure of the distilled water group remained high. The concentration of serum calcium in the Shijueming (*Concha Haliotidis*) and the distilled water groups lowered ($P<0.05$). Expression of CaL-α1C mRNA in the nifedipine group decreased compared with the distilled water group ($P<0.01$). There was the decreasing trend in the Shijueming (*Concha Haliotidis*) group, but it was not statistically significant. Shijueming (*Concha Haliotidis*) also had effects on the expression of PMCA1 mRNA but without statistical significance. However, there was a significant decreasing effect on vascular smooth muscle cell I_{Ca}-L flow.

CONCLUSION: This study indicated that Shijueming (*Concha Haliotidis*) could increase serum calcium and decrease blood pressure. It may work by influencing calcium channels, expression of PMCA1 mRNA, and regulating ion calcium channels and calcium-ATPase.

Key words: *Concha Haliotidis*; Calcium channels; Rats, inbred SHR; Plasma membrane calcium-transporting ATPases

INTRODUCTION

Ca^{2+} plays a central role in a number of important physiological processes determining vascular smooth muscle function and hypertension.[1-4] In hypertension patients, the concentration of serum calcium is low. This suggests that Shijueming (*Concha Haliotidis*) could lower blood pressure. However, the epidemiological literature suggests that the calcium and potassium content of the diet have a negative correlation with the blood pressure.[5]

Clinical studies have also shown that calcium supplementation has a minimal effect on lowering blood pressure.[6,7] A meta-analysis of over 4500 patients showed an average of 1.4 mm Hg reduction in the systolic blood pressure with calcium supplementation.[8] All analyses indicated that calcium and magnesium supplements were unlikely to lower blood pressure in adults with high-normal diastolic blood pressure. Subgroup analyses, used to formulate hypotheses, raise the possibility of a benefit to white women, which requires testing in future trials.[9] Long-term dietary cholecalciferol-calcium supplementation reduces the odds of falling in ambulatory older women by 46%, and in less active women by 65%. Supplementation had effects in men independent of their physical activity level.[10] Calcium supplementation of 1 g/day does not produce biologically significant effects on body weight. Moreover, its hypotensive effect is small and transient in women.[11] Overall, the decline in blood pressure is so small that the clinical benefit is limited.

Shijueming (*Concha Haliotidis*) is the pharmaceutical name for abalone shell which is composed of $CaCO_3$, it is commonly used in Traditional Chinese Medicine. We studied whether oral Shijueming (*Concha Haliotidis*) use has any effect on hypertension and its mechanism in spontaneously hypertensive rats.

METHODS

Experiment and procedures

Spontaneously hypertensive rats (SHR) (12 weeks old), were divided into three groups: group 1, Shijueming (*Concha Haliotidis*) (1.6 mg/mL); group 2, nifedipine (2.0 mg/mL); and group 3, distilled water. Rats were intragastrically administered drugs at a dose of 1 mL/100 g once a day for four weeks. All animal studies were approved by our institutional committee for the care and use of animals in research and education.

Blood pressure measurement

Systolic blood pressure was examined before treatment. During and after treatment, blood pressure was taken weekly.

Detection of calcium in the serum

Before and after treatment, 1.0 mL of blood was taken from SHR, and 100 μL serum was separated and stored at –20℃ until calcium determination.

Calcium channel measurement

Selected cells were placed under a microscope and the electrode was filled. Water was poured at a positive pressure (R: 2-5 MΩ) to establish an electrical potential. The electrode was gently pressed on the cell to distort minimally and establish high resistance (R>1 GΩ) with c-fast compensation. The membrane was broken by impulse negative pressure or electroshock. Capacitance was recorded, compensated with c-slow and c-series, parameters were recorded, and the ion flow picture was saved.

mRNA detection

There are 4 isomers for the plasma membrane calcium-ATPase (PMCA): PMCA1, PMCA2, PMCA3, PMCA4. The main isomer expressed in smooth muscle cells is PMCA1.[12] Therefore, we measured the expression of PMCA1. We took the pars abdominalis aortae and froze it in liquid nitrogen. We used a TOYOBO-RT-PCR kit (Toyobo, Osaka, Japan). PMCA1 PCR production was amplified to 250 bp. The primers were: forward 5′-GAAATCGCAGCCATAGTATC-3′, and reverse 5′-CTGATGACGGTGAACTTCTG-3′. CaL-α1C PCR production was amplified to 271 bp. The primers were: forward 5′-AGTGATTGCCTACGGACTAC-3′ and reverse 5′-TTGATGATGGAGTTCAGGAC-3′. β-actin PCR production was amplified to 298 bp. The primers were: forward 5′-ATGTTTGAGACCTTCAACAC-3′ and reverse 5′-GGCCATCTCTTGCTCGAAGTC-3′. The conditions for total RNA extraction were: 37℃ for 60 min, 95℃ for 10 min, and 4℃ for 5 min. CaL-α1C PCR conditions were: 95℃ for 4 min, 94℃ for 30 s, 55℃ for 30 s, 72℃ for 30 s, recycle 35 times, 72℃ for 4 min, and pause at 4℃. PMCA1 PCR conditions were: 95℃ for 4 min, 94℃ for 30 s, 58℃ for 30 s, 72℃ for 30 s, recycle 35 times, 72℃ for 4 min, and pause at 4℃.

Kodak Digital Science 1D Image Analysis Software Version 2.0.3 (Eastman Kodak, Rochester, NY, USA) and SPSS 16.0 (SPSS Inc., Chicago, IL, USA) were used to analyze data. A *t*-test was used for in-group comparison and mono-factor analysis was used for between-group comparison. Data were presented as mean ± standard deviation ($\bar{x} \pm s$). $P<0.05$ was regarded as a significant difference.

RESULTS

Effect of Shijueming (Concha Haliotidis) on blood pressure

After 4 weeks of drug intervention, the blood pressure of groups 1 and 3 had a decreasing trend compared with prior treatment. The decrease had no statistical significance ($P>0.05$). Systolic pressure in group 2 decreased significantly ($P<0.05$) (Figure 1).

After drug intervention, the serum calcium concentration in group 1 ($P<0.05$), and group 3 decreased significantly ($P<0.01$). Compared to group 3 (distilled water),

group 2 was much higher (P<0.05), and group 1 had no statistical difference with group 3 (P>0.05)(Figure 2).

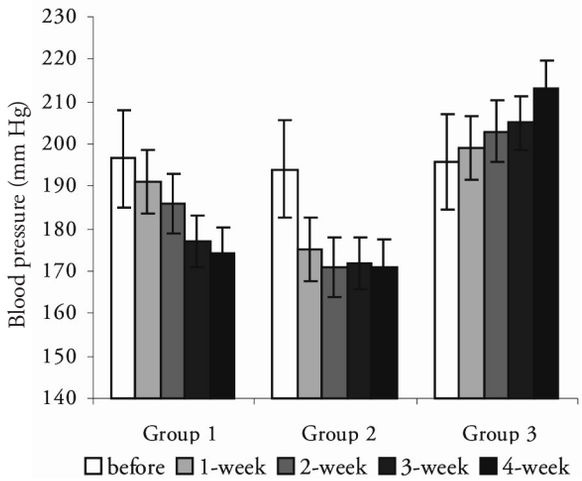

Figure 1 Comparison of blood pressure in three different groups after treatment

Group 1: Shijueming (*Concha Haliotidis*) group; group 2: nifedipine group; group 3: distilled water group.

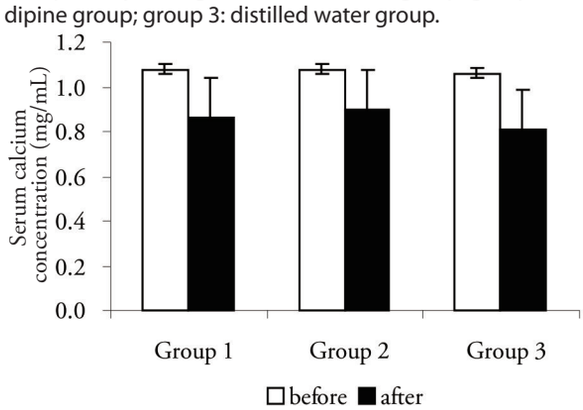

Figure 2 Comparison of serum calcium concentration among three groups

Group 1: Shijueming (*Concha Haliotidis*) group; group 2: nifedipine group; group 3: distilled water group.

Effects on calcium channels

At the same level of pressure, the Shijueming (*Concha Haliotidis*) group I-V curve moved higher. The peak value of Shijueming (*Concha Haliotidis*) was lower than that of the control group , indicating that Shijueming (*Concha Haliotidis*) decreased the trend of I_{Ca}-L, and blocked the vascular smooth muscle L-type calcium channel. However, the difference in trends was not statistically significant (Figure 3).

As shown in Figure 4, the control group had a maximum of – 10 mV, and Shijueming (*Concha Haliotidis*) group had a maximum of –25 mV.

Expression of mRNA

The mRNA expression results showed that nifedipine could down-regulate the expression of CaL-α1C mRNA in SHR (P<0.05). Shijueming (Concha Haliotidis) could also down-regulate expression of CaL-α 1C mRNA in SHR (P>0.05). And it was statistical significance (P<0.05) in the expression of CaL-α1C mRNA levels between Concha Haliotidis and nifedipine (Figure 5, 6). Results also showed that Shijueming (Concha Haliotidis) enhanced the expression level of

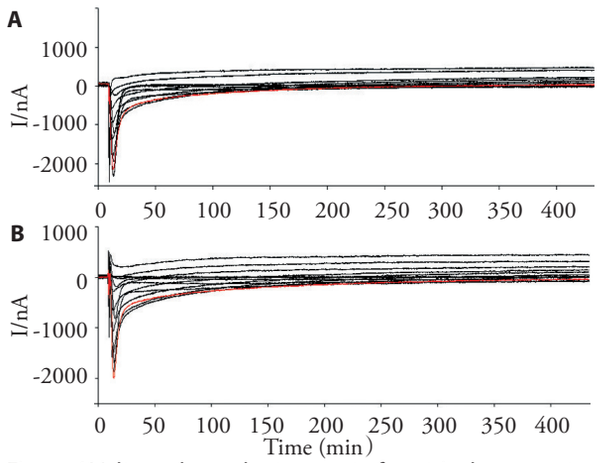

Figure 4 Voltage dependent current of ventricular myocytes

A: control group; B: Shijueming (*Concha Haliotidis*) group

PMCA mRNA in SHR with no statistical significance (P>0.05, Figure 7).

Comparison of calcium channel α1 C mRNA expression among three groups

Nifedipine downregulated the expression of CaL-α1C mRNA in SHR (P<0.05). Shijueming (*Concha Haliotidis*) also downregulated expression of CaL-α1C mRNA in SHR, but not significantly (P>0.05). There was statistical significance (P<0.05) in the difference of CaL-α1C expression between *Concha Haliotidis* and nifedipine. Expression of CaL-α1C mRNA in the nifedipine group was lower than that of the distilled water group (P<0.05), but there was no statistical difference between them (Figure 7).

Figure 8 shows that Shijueming (*Concha Haliotidis*) enhanced the expression of PMCA mRNA in SHR, but there not significantly.

DISCUSSION

Shijueming (*Concha Haliotidis*) is suggested to lower blood pressure by various mechanisms. A high calcium diet may attenuate genetic hypertension by inducing an osmotic diuresis.[13] Under normal conditions, the concentration of calcium in cells is 20 000 times lower than outside of cells.[14] There are three types of pressure-dependent calcium channels in vascular smooth muscle cells, namely L, T, and R. L-type channels, which are under calcium control, are the main channels for calcium inflow to induce vascular smooth muscle relaxation-contraction activity. It has been reported that higher calcium concentrations can strengthen the polarization of the vascular smooth muscle cytomembrane and relax muscle. This is called the membrane-stabilizing action of calcium.[15] Calcium is a key signaling molecule in determining the tension of vascular smooth muscle and the reactiveness of blood vessels. Many factors that affect blood pressure are related to calcium control. The L-type pressure-dependent calcium channel and the Ca^{2+}-ATPase on the cytoplasmic membrane are crucial to keeping calcium in balance.

We found that, as rats aged and as the concentration of

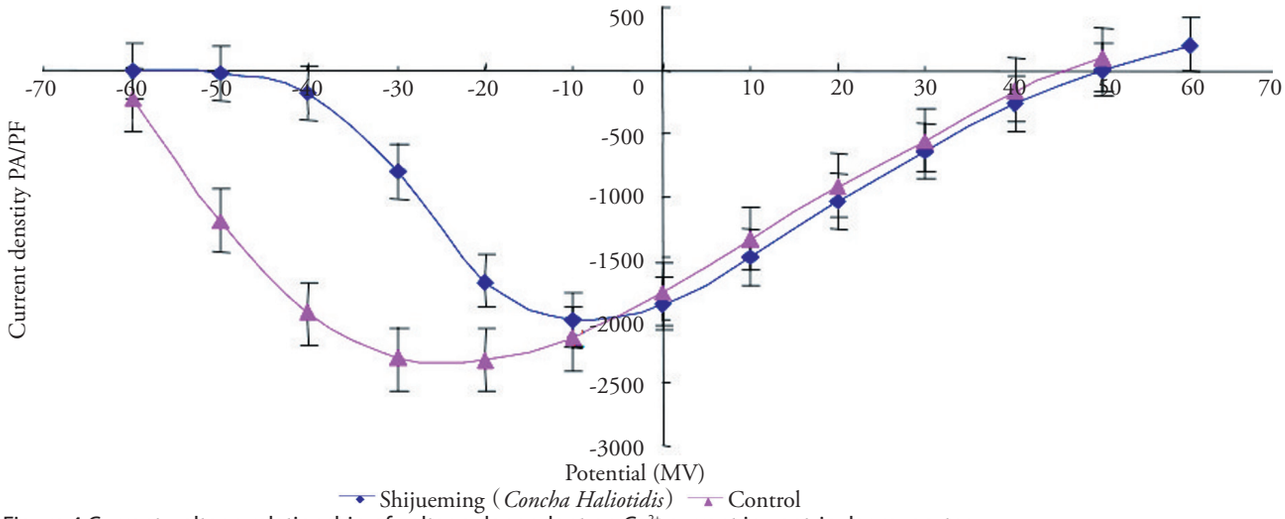

Figure 4 Current-voltage relationship of voltage dependent on Ca^{2+} current in ventricular myocytes

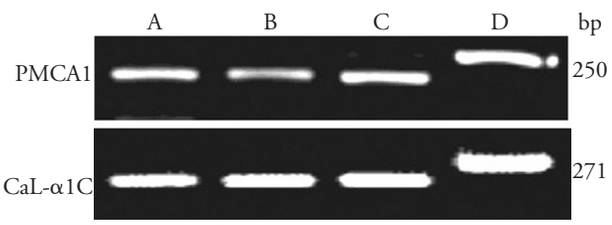

Figure 5 Expression of PMCA1 and CaL-α1C mRNA
A: Nifedipine; B: Shijueming (*Concha Haliotidis*); C: Distilled water; D: β-actin; PMCA1: plasma membrane calcium-ATPase; CaL-α1C: calcium channel α1 C.

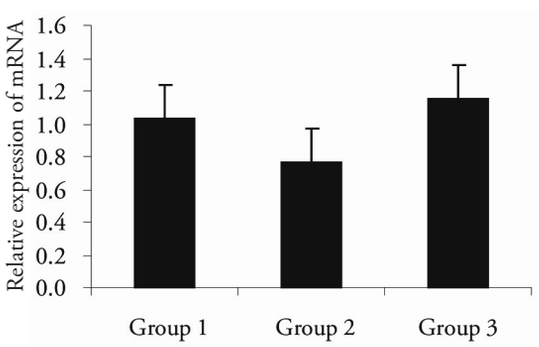

Figure 6 Comparison of plasma membrane CaL-α1C mRNA among three groups
Group 1: Shijueming (*Concha Haliotidis*); group 2: nifedipine; group 3: distilled water. CaL-α1C mRNA: calcium channel α1 C mRNA.

serum calcium in SHR decreased, blood pressure rose obviously. After the intervention of Shijueming (*Concha Haliotidis*), serum calcium rose, and blood pressure had declined. However, neither had statistical significance.

After a 4-week treatment, the calcium concentration in the nifedipine group had no significant decline, which is consistent with the literature.[16] The serum from the nifedipine group can decrease inflow of vascular smooth muscle cell I_{Ca}-L, and depress vascular smooth muscle cell I_{Ca}-L. This indicates that nifedipine has an evident inhibiting effect on L-type calcium channels.[17]

After a 4-week treatment, serum calcium in the Shijueming (*Concha Haliotidis*) group was lower, indicating that Shijueming (*Concha Haliotidis*) could block the inflow of vascular smooth muscle cell I_{Ca}-L. High calcium levels blocking L-type calcium channels could explain the mechanism of Shijueming (*Concha Haliotidis*) in decreasing the peak value of vascular smooth muscle I_{Ca}-L.

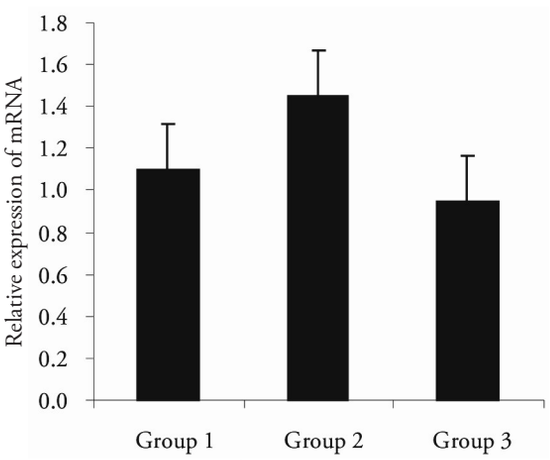

Figure 7 Comparison of plasma membrane calcium-ATPase (PMCA1) mRNA among three groups
Group 1: Shijueming (*Concha Haliotidis*) group; group 2: nifedipine group; group 3: distilled water group. PMCA1: plasma membrane calcium-ATPase.

During hypertension, the transportation, application, and metabolism of calcium are abnormal. As current of the L-type calcium channel increases, the opening of the channel is faster and inactivity is slower. This makes the opening of the channel longer and sensitive to calcium so expression is not lowered. Negative feedback control of calcium is therefore weakened.[18] Shijueming (*Concha Haliotidis*) can depress expression of CaL-α1C mRNA, decrease calcium inflow, up-regulate expression of PMCA1 mRNA, and improve calcium overloading in smooth muscle cells during hypertension to expand vessels and decrease blood pressure.

In conclusion, this study showed that, through use of Shijueming (*Concha Haliotidis*), serum calcium increased and blood pressure declined. It may work by influencing expression of PMCA1 mRNA and regulating ion calcium channels and calcium-ATPase.

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